昆 虫 学 报 *Acta Entomologica Sinica*, August 2009, 52(8): 885 – 894 ISSN 0454-6296

Origin and genetic diversity of grape phylloxera in China

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Abstract: In order to investigate levels of genetic variation and differentiation of grape phylloxera, *Daktulosphaira vitifoliae* Fitch in China, we sequenced the mitochondrial DNA (mtDNA) gene cytochrome oxidase Ⅰ (COⅠ) of 35 samples from four populations. Of the total analyzed sites, 29 (6.13%) were polymorphic, including 17 (3.59%) parsimony informative and 12 (2.54%) singleton sites. Nucleotide frequencies of A, C, G and T were 34.8%, 15.8%, 10.2%, and 39.2%, respectively. In total, 13 haplotypes were identified in the target region. The most common haplotypes were H3 (HAP-A) shared by 5 samples from SHJ, and H13 (HAP-C) found in 19 samples from SXX, HNH and LNX populations. The SHJ population was unique and shared no haplotypes with the other three populations. *Nm* ranged from 0.02 to 4.03 and the genetic distance varied from 0.001 to 0.040 between populations. *Nm* (0.02) was smaller, and the genetic distance (0.039 – 0.040) larger between SHJ and other three populations. Phylogenetic analysis and haplotype network showed that all haplotypes from SHJ formed one cluster, and the other haplotypes from SXX, HNH and LNX are grouped into another, suggesting that there were at least two introductions of grape phylloxera into China.

Key words: grape phylloxera; mtDNA COⅠ; haplotype; genetic diversity; phylogeny

1 INTRODUCTION

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is a gall-forming aphid species (*sensu lato*) native to North America and feeds on grapevine (*Vitis* spp.). The insect pest was inadvertently introduced into Europe in the 1850s, probably on the grapevine material collected for breeding resistance to powdery mildew, and its feeding on the highly susceptible roots of European grapevines (*Vitis vinifera* L.) quickly led to widespread destruction of vineyards (Ordish, 1972). It subsequently spread rapidly to vineyards around the globe and it is now found in almost all viticultural regions of the world and continues to cause economic damage (Granett *et al.*, 2001; Downie, 2005).

Grape phylloxera was first observed in China in 1892, and was subsequently observed in parts of Yantai of Shandong Province, Gaixian of Liaoning Province, Yangling of Shaanxi Province, and other places (Li *et al.*, 1992). However, because the area infected by grape phylloxera was small and grape phylloxera was controlled effectively, it did not cause great economic loss and own-rooted grapes could be cultivated all over China. However, grape phylloxera infestations have been found in some regions of China in recent years and may rapidly spread to other regions because no strict quarantine restrictions were implemented on grapevine materials, and grapevine seedlings can be freely moved between uninfested and infested vineyards (Ye *et al.*, 2006; Zhang *et al.*, 2006). Grape phylloxera populations are most damaging on the mature roots of susceptible grape strains where they create galls (tuberosities) that swell and crack, allowing entry of soil-borne fungi, which decay large portions of the root system and lead to eventual vine death (Omer *et al.*, 1999). Once a vineyard is infested it can be expected to cease production in about 2 – 5 years (Granett *et al.*, 1996). In our survey, both grapevine death and reduced yield were observed in grape phylloxera infested vineyards. Further, the resistance to the pest of several grape

基金项目: 国家自然科学基金项目(30871680);农业部2007年公益性行业(农业)科研专项(nyhyzx07-27)

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收稿日期 Received: 2009-03-09; 接受日期 Accepted: 2009-06-09

varieties, rootstocks and Chinese wild grapes were evaluated (Du *et al.*, 2008), and unfortunately, there were no resistant strains in grape varieties and Chinese wild grapes. Therefore, it is extremely urgent to find an effective way to control the grape phylloxera.

To reduce the impact of grape phylloxera, native American *Vitis* species have been used in selective breeding of resistant or tolerant rootstocks from the late 1800s, and this is currently the principal management strategy employed (Kocsis *et al.*, 1999; Granett *et al.* 2001). However, intrinsic virulence of grape phylloxera strains has been observed on feeder roots of rootstocks (Ritter *et al.*, 2007), of which a typical example was a strain or strains of phylloxera (termed biotype B) identified in California, capable of killing vines grafted on AxR#1 (Granett *et al.*, 1985). These findings stimulated molecular studies (Fong *et al.*, 1995) to analyze genetic structure (Lin *et al.*, 1999, Forneck *et al.*, 2000, Corrie *et al.*, 2002), and the origin and evolution (Downie, 2002, 2004, 2005) of grape phylloxera.

In order to control grape phylloxera, it is very important for us to understand the insect's biology, ecology and genetics. Researches on grape phylloxera have been rare in China, although it has been found in this country for more than 100 years (Lu *et al.*, 2008). Since China's economic reform and opening up to the outside world began in the late 1970s, many table grape varieties of own-rooted vines were introduced into China from Japan, United States, Italy, and France, and grape phylloxera was observed in regions where table grapevines are cultivated. Because no strict quarantine restrictions were implemented, grapevine seedlings with grape phylloxera were taken to other regions freely, which allowed the insect to spread cosmically. This chaotic situation has made management of this pest especially difficult. Information about the origin of invasive pest species often can provide some insights into their biology and potential impact (Downie, 2002). Fortunately, population genetic analysis of mitochondrial DNA (mtDNA) sequences can offer an excellent opportunity to analyze genetic differentiation and trace the genealogy of introduced organisms (Downie, 2002). Using this method, Downie (2005) suggested that there were at least two introductions of grape phylloxera into South African vineyards. In this study, we acquired mtDNA CO Ⅰ sequences for 35 samples from four grape phylloxera populations in China and analyzed the population structure, origin, and evolution of Chinese grape phylloxera.

2 MATERIALS AND METHODS

2.1 Sampling and DNA extraction

Samples of grape phylloxera were collected from grapes in 4 regions, *i. e.*, Shanghai, Hunan, Shaanxi, and Liaoning. Additional sampling details are given in Table 1. Two to three vineyards at least 500 m apart were selected in each infested region, and three to six vines with phylloxera were sampled in every vineyard. The distance separating sampled vines within each vineyard ranged from 10 – 50 m. Grape phylloxera was identified according to Granett *et al.* (2001). Samples were collected by taking galled roots from infested grape vines and kept at about 10℃ until being used for detecting galls in the laboratory. Because of the occurrence of ameiotic parthenogenesis in grape phylloxera (Morgan, 1909) and the difficulty in distinguishing eggs of different maternal origins within a gall through RAPD analysis (Lin *et al.*, 1999), the adult and its eggs within a gall are therefore considered as a single genotype as in Downie *et al.* (2001) and Downie (2002, 2005), and were pooled to comprise a sample for DNA extraction. In some cases, a few nymphs from the same root gall were combined to form a sample. Each sample was taken from a different vine. The samples were stored at −80℃ until DNA extraction was performed.

DNA extraction followed the protocol of Lin and Walker (1996) and Downie *et al.* (2000).

2.2 PCR amplification and DNA sequencing

PCR reactions were carried out to amplify a 473 bp fragment of the mitochondrial gene cytochrome oxidase Ⅰ (CO Ⅰ) described by Downie *et al.* (2001) using the primers C1-J-1718 (5′-GGAGGA TTTGGAAATTGATTAGTTC-3′) and C1-N-2191 (5′-C CCGGTAAAATTAAAATATAAACTTC-3′) (Simon *et al.*, 1994). PCR amplifications were carried out in 25 μL total reaction volumes using 1 × reaction buffer, 0.2 mmol/L dNTPs, 1 unit Taq polymerase (TaKaRa Biotech, Dalian), 2 mmol/L $MgCl_2$, 0.2 μmol/L each primer (Sangon Company, Shanghai), and 40 ng DNA template. Cycling parameters were: 2 min at 94℃, followed by 35 cycles of 30 s at 94℃, 1 min at 52℃, and 2 min at 72℃. PCR products were separated on 1% agarose gels (electrophoresis apparatus, Beijing Liuyi Instrument Factor, Beijing) and visualized on a UV illuminator after ethidium bromide staining.

PCR products were purified using TaKaRa PCR Purification Kit (TaKaRa Biotech, Dalian) and

sequenced on an ABI 3730 XL DNA Analyzer in both directions (Biosune Biotech, Beijing).

Table 1 Collection data for samples of grape phylloxera

Region	Vineyard code	Cultivar	n	Code	Haplotype	Collecting date
Malu, Jiaqing, Shanghai (SHJ)	A	Kyoho	1	SHJ1	H1	2007.08
		Centennial Seedless	1	SHJ2	H3	
		Fujiminori	1	SHJ3	H3	
	B	Kyoho	1	SHJ4	H3	2008.08
		Jumeigui	1	SHJ5	H3	
		Aagusta	1	SHJ6	H2	
	C	Yatomi Rosa	1	SHJ7	H3	2008.08
		Fujiminori	1	SHJ8	H4	
		Fujiminori	1	SHJ9	H5	
Baqiao, Xi'an, Shaanxi (SXX)	A	Kyoho	3	SXX1 – 3	H13, H6	2007.10
	B	Centennial Seedless	2	SXX4 – 5	H13, H7	
		Hutai	2	SXX6 – 7	H8, H13	
Qiancheng, Huaihua, Hunan (HNH)	A	Kyoho	3	HNH1 – 3	H13	2007.04
		Vitis davidii Foex	2	HNH7 – 8	H13, H9	
	B	Kyoho	3	HNH4 – 6	H13	2007.07
		Vitis davidii Foex	3	HNH9 – 11	H10, H13	
Xingcheng, Huludao, Liaoning (LNX)	A	Kyoho	2	LNX1 – 2	H13	2007.10
		Jing Ya	2	LNX 4 – 5	H13, H11	
		Fujiminori	1	LNX 8	H13	
	B	Kyoho	1	LNX 3	H13	2008.04
		Beta	2	LNX 6 – 7	H13, H12	

All samples were from own-rooted vines with the exception of the rootstock Beta from Xingcheng, Liaoning. n = Number of samples from each cultivar.

2.3 Data analysis

The Chinese grape phylloxera samples were treated both separately and together with 85 sequences previously collected from the native range of grape phylloxera and vineyards in 11 countries on five continents (Downie *et al.*, 2001; Downie, 2002).

Sequences were aligned in Clustal X ver. 1.83 (Thompson *et al.*, 1997). The alignment was manually edited and truncated to 473 bp using Bioedit 7.0 (Hall, 1999). Then DnaSP 4.20 (Rozas and Rozas, 1999) was used to calculate haplotype diversity, nucleotide diversity, and to execute analysis of nucleotide divergence (Dxy), net nucleotide divergence Da [$Da = Dxy - (Dx + Dy)/2$] (Nei, 1987) and gene flow (Nm) among the four grape-growing regions. MEGA 4.0 (Tamura *et al.*, 2007) was used to analyze base composition, variable sites and genetic distance by Kimura 2-paramter model.

The alignment was edited to 438 bp to match the length of sequences retrieved from GenBank (AF307429, AF307406, AF307439, AF307438). TCS ver. 1.18 (Clement *et al.*, 2000) was used to create a statistical parsimony network from the 35 Chinese samples. In addition to building a network based on a probabilistic model in which haplotypes are only connected at the 95% level of probability, TCS computes the probability of a haplotype being ancestral based on its frequency and mutational connections to other haplotypes in the sample.

Aligned sequences were also used for reconstruction of phylogenetic relationship among the included samples using PAUP* Version 4.0b8 (Swofford, 2003). The Kimura 2-parameter distance was employed to construct a neighbour-joining tree (NJ). Statistical support for nodes was estimated from 1 000 bootstrap replicates.

All sequences collected for this study have been deposited in GenBank (accession numbers: FJ493588 – FJ493622). Additional sequences from Downie *et al.* (2001) and Downie (2002) were included in the phylogenetic analysis.

3 RESULTS

3.1 Genetic variation and polymorphism of mtDNA CO I

The lengths of all sequences examined were 473 bp, therefore there were no length variations in the sequences observed within or among the test populations. The CO I sequence was strongly AT biased (A = 0.348, T = 0.392, C = 0.158, G = 0.102). There were 29 variable bases (6.13% of the total analysis sites), including 17 (3.59%) parsimony informative and 12 (2.54%) singleton sites. The parsimony informative sites were 41, 68, 137, 143, 161, 197, 251, 266, 287, 308, 350, 357, 392, 428, 443, 450, and 470. The singleton sites were at 25, 36, 74, 86, 120, 125, 186, 239, 395, 429, 446, and 455. Transitions were more

common than transversions in nucleotide polymorphic sites (Table 2).

Table 2 Variable sites in mtDNA CO I and the distribution of haplotypes in Chinese grape phylloxera populations

Haplotype	Variable nucleotide sites			Number of samples with a particular haplotype				Total occurrence of haplotypes
	1111 2346782234 5618460573	1122223333 6956880355 1716678907	334444444 992244557 258936050	SHJ	SXX	HNH	LNX	
H1	ACGCTAAATC	CTCAACATCA	TAAGTATTC	1				1
H2	T.....	.....	.G.....	1				1
H3 (HAP-A)[a]	T.....	.....	.....	5				5
H4	T.....	.....C..	.....	1				1
H5	T...C.....	.....	.....	1				1
H6	TAAT....CT	TCTG.TG.TG	C.GTC.C.T		1			1
H7	T.AT..G.CT	TCTG.TG.TG	C.G.C.C.T		1			1
H8	T.AT....CT	TCTGGTG.TG	C.G.C.C.T		1			1
H9	T.AT....CT	TCTG.TG.TG	C.G.CGC.T			1		1
H10	T.AT...GCT	TCTG.TG.TG	C.G.C.C.T			1		1
H11	T.AT....CT	TCTG.TG.TG	C.G.C.CCT				1	1
H12	T.AT.G..CT	TCTG.TG.TG	C.G.C.C.T				1	1
H13 (HAP-C)[a]	T.AT....CT	TCTG.TG.TG	C.G.C.C.T		4	9	6	19

[a] Haplotypes found in Downie (2002).

Thirteen haplotypes, two of which included multiple samples (H3 and H13, Table 2), were found among these 35 samples. H13 was shared by 19 samples from population SXX, HNH and LNX, H3 was shared by 5 samples from SHJ. Within any given sampling location, three to five haplotypes were identified. The SHJ population had the greatest number of haplotypes (5), followed by the SXX (4) and HNH and LNX population (3) (Table 3). In addition, there were no shared haplotypes between SHJ and the other three populations (Table 2).

Haplotype diversity (*Hd*) was 0.561 ± 0.178 (mean ± *SE*); nucleotide diversity (π) ranged from 0.07 to 0.22. *H*d was the greatest (0.722 ± 0.159) in SHJ, and π was the highest (0.0022) in SXX, however these two parameters were the smallest in the HNH population. None of the sampling locations showed a significant deviation from neutrality as indicated by Tajima's *D* statistic (Table 3).

Table 3 Haplotype diversity, K and π of CO I in four grape phylloxera populations

Population	Sample size	Number of haplotypes	% Variable sites	Haplotype diversity	K[a]	π[b]	Tajima's *D*
SHJ	9	5	0.44	0.722 ± 0.159	0.8889	0.0017	−1.6094
SXX	7	4	0.57	0.714 ± 0.181	1.1429	0.0022	−1.4341
HNH	11	3	0.18	0.345 ± 0.172	0.3636	0.0007	−1.4693
LNX	8	3	0.25	0.464 ± 0.200	0.5000	0.0010	−1.3101

[a] K = average number of differences; [b] π = nucleotide diversity.

Dxy, the average number of nucleotide substitutions per site between two populations, is a measure of nucleotide diversity. *Dxy* and net nucleotide divergence (*Da*) were estimated according to Nei (1987) and Tajima (1983) (Table 4). The range of *Dxy* between the four grape phylloxera populations was 0.159% − 3.809%, and *Da* was 0.00% − 3.59%. *Dxy* between SHJ and the

other three populations was relatively large; however, it was small between the other three populations. The smallest *Dxy* was between HNH and SXX closely followed by HNH and LNX. The net divergence was relatively low between four grape phylloxera populations, with the highest values between the SHJ and the other three populations (0.03594). However, there was no difference for *D*a between the other three populations.

Table 4 Interpopulation nucleotide divergence (*Dxy*) (the upper triangle) and interpopulation net nucleotide divergence (*Da*) (the lower triangle) in grape phylloxera

	SHJ	SXX	HNH	LNX
SHJ	*	0.03809	0.03726	0.03741
SXX	0.03594	*	0.00159	0.0174
HNH	0.03594	0.0000	*	0.0091
LNX	0.03594	0.0000	0.0000	*

3.2 Gene flow and Kimura 2-parameter distance

Gene flow (*Nm*) into a population can counteract gene frequency changes because of selection, imposing a limit on local adaptation. *Nm* between the four grape phylloxera populations was measured by DnaSP 4.20 and ranged from 0.02 to 4.03 (Table 5). *Nm* between HNH and LNX was largest (4.03), followed by that between HNH and SXX (3.12), which indicated that gene flow was higher among SXX, HNH and LNX, and smallest between SHJ and any of the other populations (0.02).

Kimura 2-parameter pairwise genetic distances, based on the COⅠ sequences, were very small within populations (0.001 – 0.002). However average distances varied from 0.001 to 0.040 between populations (Table 5). The genetic distance between SHJ and SXX was the greatest, and the distance between HNH and LNX was the smallest. In addition, the genetic distance calculated for SHJ and three other populations (SXX, HNH and LNX) indicated that they were substantially different, but was nearly identical among these three populations (0.001 – 0.002), and equal to distances within populations.

Table 5 *Nm* (the upper triangle) and Kimura 2-parameter distances (the lower triangle) between populations of grape phylloxera

	Within population	SHJ	SXX	HNH	LNX
SHJ	0.002	*	0.02	0.02	0.02
SXX	0.002	0.040	*	3.12	3.07
HNH	0.001	0.039	0.002	*	4.03
LNX	0.001	0.039	0.002	0.001	*

3.3 Phylogenetic relationships among grape phylloxera populations

In the statistical parsimony network all samples clearly grouped into two distinct clades (Fig. 1). Nine samples were grouped in Clade 1, and all of them were from the SHJ region. The 26 samples from the other three populations were grouped in Clade 2. Five samples fell into Haplotype A (H3), all of them were from the SHJ region. Haplotype C (H13) was represented by 19 samples as well, which was a mix of samples from SXX (n = 4), HNX (n = 9) and LNX (n = 6) populations (Table 6).

Table 6 Haplotype list for grape phylloxera sampled from Chinese vineyards

Haplotype	Included samples
HAP-A (H1)	SHJ2, SHJ 3, SHJ 4, SHJ 5, SHJ 7
HAP-C (H13)	SXX1, SXX3, SXX4, SXX7, LNX1, LNX2, LNX3, LNX4, LNX6, LNX8, HNH1, HNH2, HNH3, HNH4, HNH5, HNH6, HNH7, HNH10, HNH11

Unique haplotypes are coded as in Table 1. Haplotypes A and C were found in Downie (2002).

The neighbor-joining (NJ) analysis showed that the 13 haplotypes of Chinese grape phylloxera were placed into two clusters (Fig. 2). All the haplotypes of SHJ fell into one cluster (Clade 1), the other eight haplotypes from SXX, HNH and LNX were grouped into another (Clade 2). The Clade 1 haplotypes were most closely related to the haplotypes from New Zealand, Australia and California in USA, which were most closely related to the haplotypes of native grape phylloxera from *V. vulpina* (L.) in Virginia (Fig. 2). The Clade 2 haplotypes were most closely related to haplotypes from Oregon, Washington and Australia. In addition, this clade was most closely related to haplotypes of native grape phyloxera from *V. riparia* (L.) in New York.

In addition, the results showed that there were no correlation between grape phylloxera haplotypes and the host varieties. Grape phylloxera haplotyes from the same host variety in the same sample site may not be the same, whereas the phyloxera haplotypes from different varieties may be the same (Table 1). Only one or two base pair difference exists between haplotypes in the same clade, while 17 – 19 bp differences are found between haplotypes from different clades (Fig. 1). These results suggest that differentiation within each of the two Chinese haplotype groups is weak, but is substantial between them.

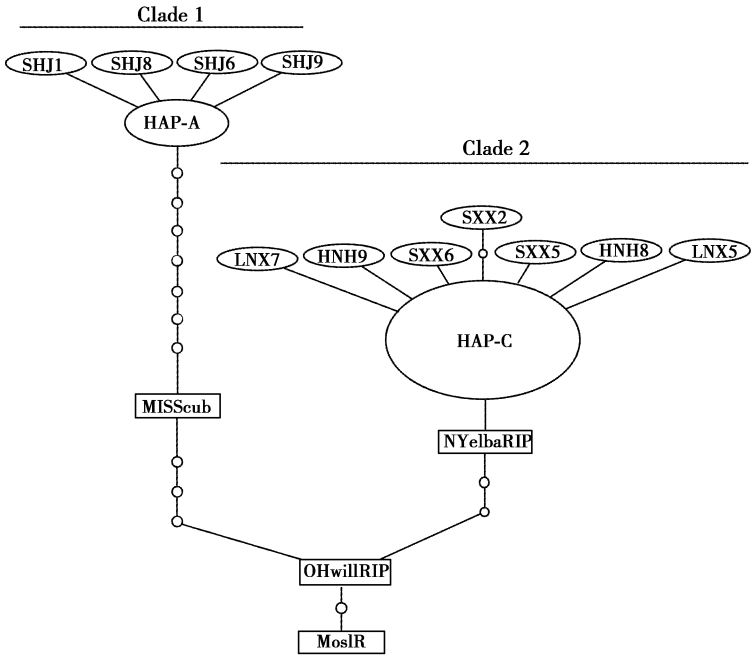

Fig. 1 Haplotype network of grape phylloxera CO I sequences collected in China

The size of the circles is proportional to the number of included haplotypes; the very small circles represent mutations separating each haplotype (unsampled haplotypes); the larger rectangles indicate haplotypes that were acquired from GenBank, and they were treated as the root of tree.

4 DISCUSSION

In this survey, genetic variation and differentiation of Chinese grape phylloxera were analyzed based on mtDNA CO I sequences. Divergence between SHJ and the other three populations was large, while no differentiation was found among the other three populations (Table 4). Analysis based on genetic distances revealed that low levels of genetic variation were found in the SXX, HNH, LNX populations, while SHJ was significantly different from the other three populations. In addition, Haplotype C was shared by 19 samples from SXX, HNH, LNX populations, but there were no shared haplotypes between SHJ and the other three populations, which means that they may come from different ancestors.

In population genetics, restricted gene flow, genetic drift, and natural selection are the main causes of population differentiation (Zheng *et al.*, 1997), whereas gene flow can reduce the genetic differentiation between populations (Lenormand *et al.*, 1998). When $Nm > 1$, genetic differentiation may be prevented (Wright, 1931). Here, Nm (3.07 – 4.03) was larger among SXX, HNH, LNX populations, which indicate higher levels of gene flow and less genetic differentiation between these populations. In addition, Haplotype C was found at a high frequency in all three populations and the genetic distances (0.001 – 0.002) were very small among them. Given the fact that the three regions are very far apart (> 1 000 km) and that parthenogenesis is the primary reproductive mode of Chinese grape phylloxera, it seems likely that the higher estimates of Nm represent historical gene flow in the native range and lack of differentiation among these populations is caused by man-made introduction rather than by wind dispersal or migration of asexual populations.

Host plant species have been reported to be an important factor influencing adaptation of races or demes in aphids (Kimberling and Price 1996). Corrie *et al.* (2003) used SSR markers to identify strong associations between asexual lineages and host types in Australian vineyards. However, we found no association between phylloxera lineages and a given host variety for the grape phylloxera populations included in this study. Three factors may be responsible for this phenomenon, *i. e.*, the primary mode of reproduction of Chinese phylloxera is parthenogenesis, the host varieties are all equally susceptible to grape phylloxera in China, and the vineyards infested by grape phylloxera have not been established for a very long time. Therefore, the genetic differentiation of grape phylloxera in China is most likely to be primarily affected by their origin.

Haplotype diversity was substantial: 13 haplotypes were found among the 35 samples of grape phylloxera from Chinese vineyards (Table 2).

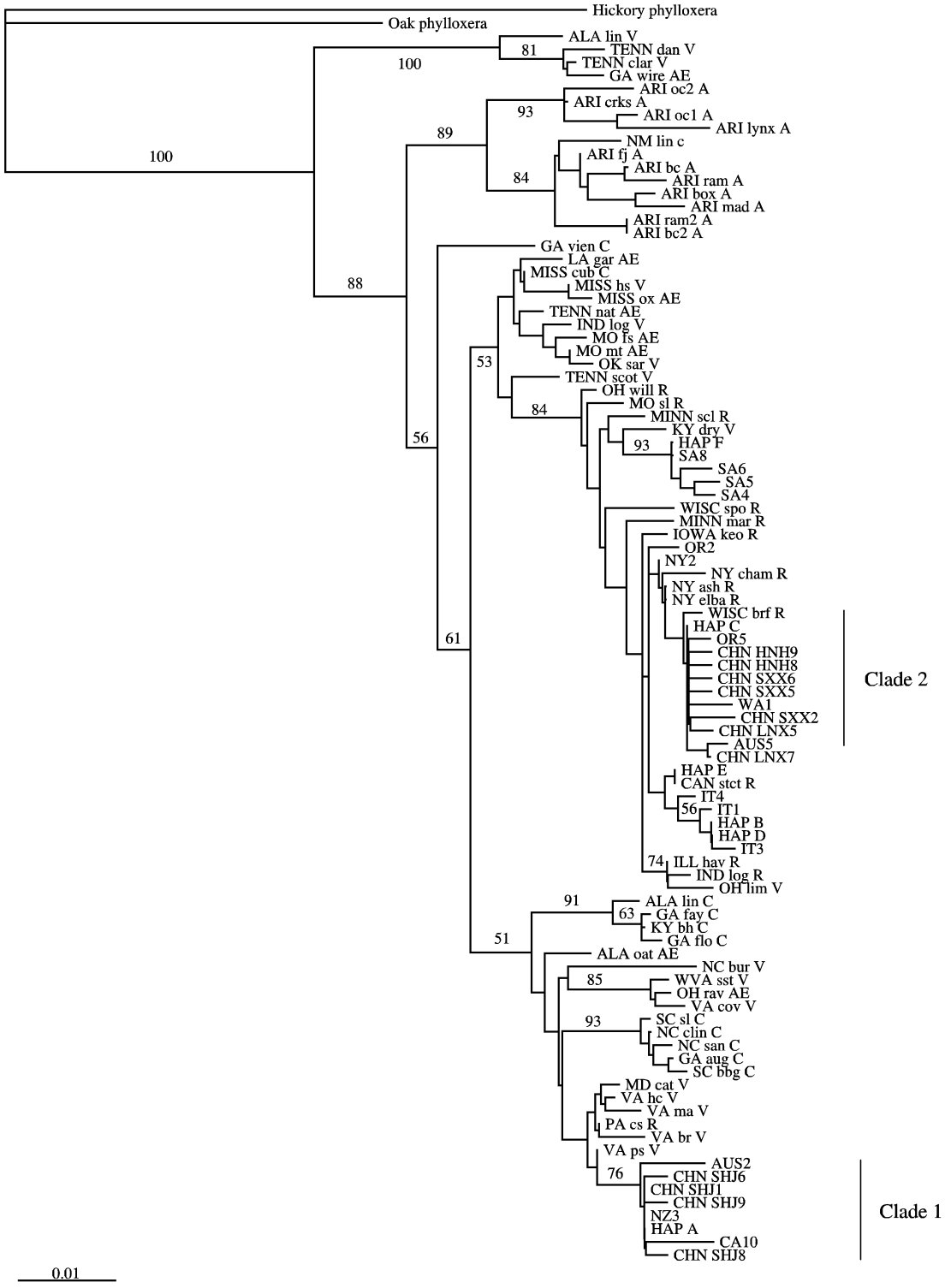

Fig. 2 Gene genealogy of all haplotypes sampled in both native and introduced ranges globally, showing placement and relationships of Chinese haplotypes

Grape-growing countries are abbreviated as follows: NZ = New Zealand, AUS = Australia, CAN = Canada, IT = Italy, CHN = China. California is abbreviated for CA. Composite haplotypes contain samples as follows: HAP-A: China, California, Peru, New Zealand, and Australia; HAP-B: California, France, Germany, Hungary, Italy, Australia; HAP-C: China, Pennsylvania (native), United Sates, Canada, France, Germany, Australia, New Zealand, and Argentina; HAP-D: France, Hungary; HAP-E: Canada, France; HAP-F: South Africa; HAP-G: South Africa. Native american haplotype abbreviations are state, locality, and *Vitis* species (V = *V. vulpina*, A = *V. arizonica*, R = *V. riparia*, C = *V. cinerea*, AE = *V. aestivalis*). Further details can be found in Downie *et al.* (2001) and Downie (2002). Numbers indicate bootstrap support from 1 000 replications when > 50%.

Previous studies (Downie, 2002; Corrie *et al.*, 2003; Downie, 2005) have sampled 20 haplotypes from commercial vineyards located in several countries, of which only six were observed multiple times (haplotypes A – F). Of these six haplotypes, only A and C were identified in the present study; 11 of our 35 samples had unique haplotypes, although these haplotypes generally differed from the more common haplotypes by only a single or two mutational steps (Fig. 1). All haplotypes were divided into two clades (Figs. 1 and 2), suggesting that there were at least two introductions of grape phylloxera into China.

It has been widely assumed that introductions of grape phylloxera around the world have largely been *via* France (Ordish, 1972). Downie (2002) compared CO Ⅰ sequences of samples collected from the native and introduced ranges of grape phylloxera to infer the sources and pattern of introductions in worldwide viticulture. His data suggested that most grape phylloxera in viticulture, including those from Europe, have originated in the northern USA where *Vitis riparia* dominates. There was evidence for independent introductions into Europe, South Africa and California and subsequent spread from California into Australia, New Zealand and Peru. However, the South African populations were represented by samples from a single grape-growing region in the Western Cape. Downie (2005) subsequently compared the different sources of grape phylloxera in South Africa collecting from multiple vineyards and grape growing regions, and found evidence that there were at least two introductions into that country. Similarly, Corrie (2003) provided strong evidence showing that grape phylloxera in Australia has multiple origins and falls into two clades partially related to vine host usage. Like these studies, our results strongly suggest that there were at least two introductions of grape phylloxera into China. The data suggest an ultimate origin of Clade 1 haplotypes from the southeast USA, possibly from V. *vulpina*, and Clade 2 haplotypes from the northeast USA, possibly from *V. riparia*, but what route they took to get to China is not clear. More rapidly evolving markers such as microsatellites might allow more precise tracking to sources within the introduced range where genetic diversity and structure is insufficient to allow assignment to a source using DNA sequences.

The findings of this study provided valuable information about grape phylloxera in China and could be used in monitoring and prevention of aggressive genotypes in China, although we did not have enough information to allow for a precise tracking of the sources of the Chinese grape phylloxera populations. We also believe that our results can be used as the basis for the breeding and selection of resistant grape rootstocks and for the management of grape phylloxera in infested vineyards.

ACKNOWLEDGEMENTS We thank the vineyard owners and employees who assisted in sampling of grape phylloxera in vineyards. We would be remiss not to thank our undergraduates whose collecting efforts made this work possible: DU Yuan-Peng; YANG Yang, ZHAO Qing, WANG Zhao-Shun and WANG Hui.

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我国根瘤蚜 mtDNA CO Ⅰ遗传多样性与系统发育

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摘要:通过线粒体 DNA CO Ⅰ多态性研究了我国根瘤蚜 *Daktulosphaira vitifoliae* Fitch 的遗传分化与系统发育,并将我国根瘤蚜单倍型与 GenBank 中已发表的 85 个单倍型进行了聚类分析。结果表明:序列中 A,C,T,G 4 种核苷酸的比例分别为 34.8%,15.8%,39.2% 和 10.2%, 29 个变异位点中单一多态位点 12 个,简约信息位点 17 个。确定了 13 种单倍型,检测 5 种单倍型,其中上海群体单倍型相对丰富,且上海葡萄根瘤蚜群体与其他 3 个群体之间没有共享单倍型。同时上海群体与其他 3 个群体的遗传距离最大(0.039 ~ 0.040),*Nm* 最小(0.02),在分子系统发育树和单倍型网络图上为独立分支,说明我国根瘤蚜至少有两个独立的起源。

关键词: 葡萄根瘤蚜; mtDNA CO Ⅰ; 单倍型; 遗传多样性; 系统发育

中图分类号:Q968　　**文献标识码**:A　　**文章编号**:0454-6296(2009)08-0885-10

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